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REVIEW | *Physiological Genomics of Cell States and Their Regulation and Single Cell Genomics*

Roles of estrogen receptor-alpha in mediating life span: the hypothalamic deregulation hypothesis

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[1]*Lawrence Berkeley National Laboratories, Berkeley, California;* [2]*Center for Research and Education in Aging, University of California at Berkeley, Lawrence Berkeley National Laboratories, California; and* [3]*Department of Integrative Biology, University of California at Berkeley, Berkeley, California*

Gouw AM, Efe G, Barakat R, Preecha A, Mehdizadeh M, Garan SA, Brooks GA. Roles of estrogen receptor-alpha in mediating life span: the hypothalamic deregulation hypothesis. *Physiol Genomics* 49: 88–95, 2017. First published December 23, 2016; doi:10.1152/physiolgenomics.00073.2016.—In several species caloric restriction (CR) extends life span. In this paper we integrate data from studies on CR and other sources to articulate the hypothalamic deregulation hypothesis by which estrogen receptor-alpha (ER-α) signaling in the hypothalamus and limbic system affects life span under the stress of CR in mammals. ER-α is one of two principal estrogen-binding receptors differentially expressed in the amygdala, hippocampus, and several key hypothalamic nuclei: the arcuate nucleus (ARN), preoptic area (POA), ventromedial nucleus (VMN), antero ventral periventricular nucleus (AVPV), paraventricular nucleus (PVN), supraoptic nucleus (SON), and suprachiasmatic nucleus (SCN). Estradiol signaling via ER-α is essential in basal level functioning of reproductive cycle, sexually receptive behaviors, physiological stress responses, as well as sleep cycle, and other nonsexual behaviors. When an organism is placed under long-term CR, which introduces an external stress to this ER-α signaling, the reduction of ER-α expression is attenuated over time in the hypothalamus. This review paper seeks to characterize the downstream effects of ER-α in the hypothalamus and limbic system that affect normal endocrine functioning.

estrogen receptor; hypothalamus; HPG axis; sexual dimorphism; sexual behavior; stress response; circadian rhythms; limbic system; caloric restriction; longevity

THE HYPOTHALAMUS GOVERNS developmental processes in vertebrates, including mammals, fish, birds, reptiles, and amphibians (8, 13, 15, 16, 39). While the precise mechanism by which the hypothalamus contributes to development and aging is unknown, multiple nuclei of the hypothalamus function to modify organismal sensitivity to hormones for maintaining cellular and systemic homeostasis throughout an organism's life span (4, 39). In conjunction with key endocrine glands, such as the anterior pituitary, hormonal actions in various hypothalamic nuclei ultimately produce characteristic effects on somatic growth, aging, and longevity (36, 39, 56).

In this study, we seek to delineate the hypothalamic deregulation hypothesis of aging, in which the hypothalamus influences the process of aging and the onset of discrete developmental phases. This hypothesis postulates that hypothalamic hormonal sensitivity is enhanced under specific conditions, such as chronic stress. Altered hypothalamic sensitivity is implicated in the interruption of downstream communication with key neuroendocrine centers in the brain as well as endocrine glands throughout the body. Altered hypothalamic sensitivity to endocrine signals, in turn, results in a disruption of regular autonomic and endocrine functioning of the hypothalamus, which ultimately leads to the phenotypic effects of aging. Historically, studies show that one method of limiting the aging process is through long-term caloric restriction (CR) (14, 35, 50, 55). The hypothalamic deregulation hypothesis provides an explanation as to how CR slows aging by preventing drastic changes in the sensitivity of hypothalamic nuclei to various hormones, but most specifically estrogen.

Estrogen has two primary receptor types: estrogen receptors (ER)-alpha (α) and -beta (β) (40, 48). Extensive research has shown that these receptor types function independently and are distributed broadly yet specifically in several key regions/nuclei of the hypothalamus and in the limbic system (45, 48). In this paper, we have chosen to focus on the alpha receptor subtype. Encoded by the gene estrogen receptor 1 (ESR1) in

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humans, ER-α is composed of three domains: a modulating NH_2-terminal domain, a DNA-binding domain, and a ligand-binding COOH-terminal domain (40). To explain the hypothalamic deregulation hypothesis, we will spend the remainder of this paper focusing on the expression and functions of ER-α in a number of hypothalamic nuclei and limbic regions. We apply the principles of the hypothalamic deregulation hypothesis as a means to speculate what the ultimate effects of CR on aging via estrogen signaling may be.

There seem to be significant evolutionary implications of long-term CR in aging via ER-α in the hypothalamus and limbic system. In this review, we aim to compile the results of previous studies in the field of aging research. From these data, we propose that unique signaling pathways have evolved in several species as a result of changing food conditions. Our hypothesis is congruent with the idea that these pathways grant organisms the ability to alternate between normal and low food availability, ultimately allowing them to survive (50). Results of selected studies will be discussed in the context of such evolutionary implications. Components of our hypothalamic deregulation hypothesis along with experimental results are presented sequentially (Fig. 1).

Localized Control of Reproduction

ER-α signaling in the arcuate nucleus (ARN) and the preoptic area (POA) is primarily responsible for controlling reproductive function (45, 46, 58). More specifically, ER-α acts to facilitate normal menstrual/estrous cycle through the homeostasis of various sex hormones such as gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) (46). Disruption of ER-α signaling results in decreased LH surge during estrous cycle. ARN and POA interactions are accomplished through transsynaptic communication between estrogen-sensitive ER-α neurons in the ARN and GnRH-producing neurons in the POA (46). ER-α knockout in the mouse ARN causes acyclicity, rendering the mice in constant estrous cycle (58). These observations show the crucial function of ER-α expression in the rostral ARN in maintaining cyclicity and estrogen-dependent negative feedback. Thus ARN and POA ER-α expression regulates GnRH production to maintain normal endocrine reproductive function.

In addition to projections from select ER-α expressing neurons in the ARN, strong afferent projections appear to extend from the medial preoptic area (MPOA) to GnRH-producing neurons in the rostral POA. These neurons are thus implicated, along with other projecting neurons from the ARN, in the control of downstream GnRH production (46). Similar studies indicate that ER-α is coexpressed directly with GnRH-producing neurons in the POA, providing the strong possibility of a direct control of GnRH production via ER-α (5).

Other than manipulating expression of GnRH and LH, estrogen may also collaborate with other factors to act directly on GABAergic neurons in the POA via estrogen receptor coexpression on these neurons as a means of controlling negative feedback related to estrogen (25). In addition, several prolactin-producing neurons in the brain, including neurons in the ARN, express ER-α mRNA. It is hypothesized that estrogen regulation of prolactin production is likely achieved through posttranscriptional mechanisms (20). ER-α has also been shown to be strongly coexpressed with kisspeptin-expressing cells (kp10ir cells) in the caudate arcuate nucleus of the mouse hypothalamus. Similar to ER-α, kisspeptins are known to induce downstream production of GnRH and LH (19). Thus manipulation of GnRH and LH can be influenced by ER-α also via other factors such as GABA and prolactin.

CR has been known to preserve ER-α expression in the hypothalamus. It may initially seem counterintuitive to maintain reproductive function during CR. From a perspective of teleology, it may be supposed that if an animal is starving as under CR, the reproductive pathways should be suppressed due to the substantial energetic demands of pregnancy and childbearing. However, when reevaluated in terms of aging, ER-α

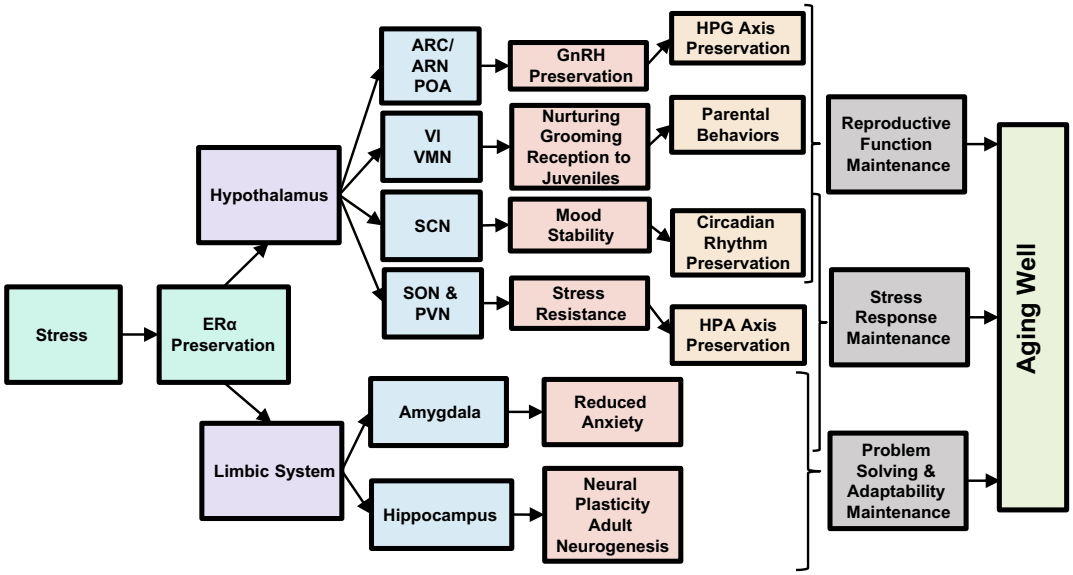

Fig. 1. Hypothesized model of how via estrogen receptor alpha (ER-α) signaling stress mediates hypothalamic and limbic system functioning to affect somatic health in aging.

preservation may make sense from the perspective of long-term animal survival. To the extent that CR extends life span, extension of an organism's reproductive functions would be a favorable outcome. From this, it is reasonable to suspect that extension of fecundity and overall life span during conditions of CR, via ER-α preservation in the hypothalamus, is likely evolutionarily selected.

Sexual Dimorphism, Sexual, Maternal, and Social Behaviors

ER-α signaling in the ARN and POA, as well as the ventromedial nucleus (VMN) and anteroventral paraventricular nucleus (AVPN) is primarily responsible for modulating sexual and social behaviors and determining differential developmental organization of neural circuitry between males and females (41).

Sexual dimorphism in ER-α expression. Studies have shown sexually dimorphic increased ER-α expression that correlates with Kiss1 expression in virtually all Kiss1-expressing hypothalamic regions in the female rat brain (AVPV, MPOA, and ARC) (19). In contrast, male rats had significantly greater ER-β expression in the AVPV at birth, but this then changes in adult age where female ER-β expression exceeds that in males. Kiss1 expression especially in the ARC was found to be the key regulatory factor in sexually dimorphic expression of ER-α in the neonatal brain (8, 57).

Such sexually dimorphic ER-α induction has been shown due to differential sexual hormone levels. Postnatal prairie voles treated with oxytocin showed that by the age of 3 wk female voles exhibited an increase of ER-α in the ventral lateral septum (LSV), ventromedial hypothalamus (VMH) and the central amygdala. Other areas, such as the MPOA, medial amygdala (MeA) or ARC did not exhibit any apparent treatment effects in either males or females (57). It was shown that oxytocin has organizational effects on ER-α expression that are sexually dimorphic. Early treatment of oxytocin induces ER-α expression, yet inhibition of oxytocin in adult age causes distinct reduction in ER-α differentially in males and females (57). The organizational effects of estradiol within the AVPV results in modest sex differences between male and female vole development.

Besides prairie voles, sexually dimorphic regulation of ER-α is also found in other rodents. For example, male and female rats naturally express different levels of ER-α in different parts of the hypothalamus partly due to the inhibition by testosterone in male rats (26). Thus, there are multiple pathways that contribute to the sexually dimorphic expression of ER-α.

A sexual dimorphic phenomenon also influences circadian rhythm. Estrogens appear to act directly on neurons of the circadian clock in suprachiasmatic nucleus (SCN) neurons. The levels of expression appear to be sexually dimorphic, with higher expression of ER-α in females than in males (29). Coexpression of ER-α and ER-β has been found in neurons and astrocytes of the SCN. Furthermore, estrogen receptor coexpression with GABAergic neurons suggests that the mechanism of estradiol signaling in the SCN may be mediated by this GABAergic signaling (48).

Sexual dimorphism of ER-α expression may suggest that CR differentially extends life span in males vs. females (26, 29, 48, 57). It could be the case that the higher expression of ER-α in females, which is preserved under CR, extends life span in females when compared with males. One could speculate that sexual dimorphism of ER-α expression may contribute to the well-known epidemiological data that show longer average life spans for women than men.

Sexual and maternal behaviors. The aforementioned sexual dimorphic ER-α expression in the hypothalamus gives rise to female sexual and parental behaviors that are less prominent in males. Inhibition of ER-α signaling results in decreased rodent maternal behaviors such as licking and grooming. The MPOA in female rodents plays a significant role in controlling maternal behaviors. In POA neurons, siRNA knockdown of ER-α reduced characteristic maternal behaviors and increased aggression toward sexual partners/intruders. These observations demonstrate a crucial and significant role of POA-localized ER-α in maintaining maternal behaviors (41).

In female rodents, it is well known that increased ER-α expression in the ventrolateral ventromedial nucleus (vlVMN), ARN, and MPOA corresponds to increased sexual behaviors and sexual receptivity to a male (7, 44, 47). First, ARN ER-α increases sexual receptivity in female rodents. ER-α forms a complex with metabotropic glutamate receptor (mGluR1a), ultimately allowing for the completion of signaling of the lordosis circuit (10). Second, in the vlVMN ER-α increases grooming and nurturing behavior. What a female rat experiences from its mother can affect ER-α expression in their vlVMN. Low grooming/nurturing offspring exhibited lower ER-α expression (7). Third, ER-α expression in the MPOA of primiparous rats may determine their ability to recover from postpartum anxiety and depression. An increase in ER-α expression in the MPOA of primiparous rats 10 wk postpartum coincided with a recovery from anxiogenic and depressive responses (21). Thus it seems that ER-α expression in vlVMN, ARN, and MPOA has various sexual and maternal behavioral effects in female rodents.

Though less prominent, male mice are also affected by hypothalamic ER-α expression. It has been shown that sexual behaviors and aggressive behaviors are governed by estradiol-organized circuitry in the MPOA and the VMH in male mice (44). Knockdown of ER-α expression in the VMH reduced both types of behaviors, whereas knockdown in the MPOA resulted only in a reduction of sexual behaviors (44). This phenotype is also seen in female mice, where knockout of ER-α in vlVMN results in increased aggression toward juveniles. Aggressive behaviors appear to be at least partially governed by estrogenic signaling via ER-α in the VMN (47).

The aforementioned studies indicate that ER-α not only hormonally increases fecundity via GnRH and LH, but that ER-α also promotes the mating and nurturing behaviors that would be necessary for successful reproduction (7, 10, 21, 44). In addition, such positive behaviors are accompanied with reduction of aggressive behaviors toward their mates and juveniles. Though such behavioral changes are mostly in females, similar behavioral changes can be seen in males as well. Overall, results are consistent with the notion that when CR extends aging, CR also promotes sustained mating and nurturing behaviors that would enable successful reproduction even in old age (7, 10, 21, 44, 47). This lends additional support to the notion that despite reduced fecundity, CR extends the window for reproduction, through the preservation of the ER-α signaling in the hypothalamus.

Stress Response and the Hypothalamic-Pituitary-Adrenal Axis

ER-α signaling in the paraventricular and supraoptic nuclei (PVN and SON) is primarily responsible for controlling an organism's response to physiological and environmental stressors, such as osmotic stress, hunger, and activation of the hypothalamic-pituitary-adrenal axis (HPA axis) (24, 50, 55, 56).

Estradiol signaling via ER-α in the PVN has been shown to affect the HPA axis. Where older rodents show a decrease in glucocorticoid receptors, estrogen treatment restores them. As a result, ER-α restores the HPA feedback loop in the presence of stressors (13). A correlative study determined that estrogen likely acts directly on corticotropin releasing hormone (CRH)-producing neurons in the PVN to affect the HPA axis (1, 6). It has been shown that reproductive experience in female rodents can determine the level of ER-α activation in regions such as the PVN and the gene expression of CRH in response to stressful circumstances, such as a maze test (6). Moreover, increased ER-α expression (in PVN, not SON) combats effects of osmotic stress (24). These findings are consistent with what is seen in humans. There is an inverse correlation between CRH and ER-α neurons in postmortem test subjects that suffered from mood disorders such as depression or bipolar disorder (1).

In addition to modulating the HPA axis, ER-α and -β are responsible for controlling the nitric oxide (NO) metabolism in the SON and PVN under basal conditions. Nitric oxide is produced by hypothalamic neurons upon osmotic stress. However, under acute osmotic stress situations (i.e., NaCl administration), NO-producing neurons are drastically reduced, especially in the SON and PVN. However, an increase of ER-α upon administration of ER-α agonists prevents the reduction of NO-producing neurons in PVN and SON (24). The response to osmotic stress demonstrates the neuroprotective roles of ER against osmotic stress in the hypothalamus.

Studies on estradiol signaling via ER-α in the PVN could indicate that ER-α has downstream effects stemming beyond the reproductive system. ER-α is also implicated in modulating the stress response. A naturally imposed CR diet results from a lack of food; thus, one could posit that ER-α function is preserved upon CR as a psychological self-defense mechanism against the stress that comes with starvation. In other words, the ability of ER-α signaling to overcome stress could be far more directly linked to CR than previously thought. The proreproductive functions of ER-α could simply be auxiliary advantages that accompany CR, which ultimately contribute to the increase in an organism's fitness.

Circadian Rhythms in the SCN

The circadian pacemaker that regulates the sleep cycle as well as biological rhythms of hormones and body temperature is localized within the SCN. These SCN neurons contain ER-α, ER-β, and progesterone receptors. ER-α signaling in the SCN is responsible for the regulation of clock gene expression and communication with neighboring hypothalamic nuclei via afferent synaptic connections to maintain time-regulated hormonal functioning (12).

In addition to ER-α and ER-β expression being directly localized in the SCN, neurons afferent to the SCN projections from the MPOA, the ARN, and the PVN also express ER-α and ER-β (12). Hence, the pattern of ER isoform expression provides further evidence for estrogenic signaling to the SCN (12). Double label immunocytochemistry for ER-α and ER-β and CtB (retrograde tracer cholera toxin B subunit) has shown that estrogen regulates the circadian rhythm through steroid-binding systems afferent to the SCN. This indicates that neuroanatomical pathways exist afferent to the SCN that mediate effects of ER-α and ER-β on the circadian rhythm.

ER-α regulation of the circadian rhythm would be another way by which CR promotes normal circadian functioning upon aging (12). Dysfunctional SCN and disrupted circadian rhythms are observed in older patients with dementia, as well as in postmenopausal women (18, 39). More interestingly, estradiol is able to restore normal SCN functioning in postmenopausal women. Since disruption of circadian rhythm is common in old age, increased ER-α and ER-β expression and function would need to be preserved to allow for normal circadian rhythmicity. As CR has been shown to extend life span, we would expect CR individuals to have especially active estrogen receptors in their phenotypes.

Expression in the Limbic System: ER-α Signaling in the Hippocampus and Amygdala

In addition to broad ER-α expression within the aforementioned nuclei of the hypothalamus, estradiol signaling via ER-α has also been observed in the limbic system, primarily in the amygdala and the hippocampus (40). In the adult rat hippocampus, ER-α expression is detected in the interneurons of the dentate hilus polymorphic region, and in the CA1 stratum radiatum (40). Since ER-α is also expressed in inhibitory neurons of the CA1 region of the hippocampus, estradiol signaling preserves neural plasticity of the hippocampus (40).

Like in the hypothalamus, ER-α has neuroprotective effects in the hippocampus. Within the neurons of the hippocampus in vitro, estrogens, including those that signal specifically through ER-α, shield against glutamate toxicity, glucose deprivation, $FeSO_4$ toxicity, and amyloid-beta peptide toxicity, the last of which contributes to Alzheimer's disease (23). Learning and memory in the hippocampus are preserved by the induction of adult neurogenesis in the dentate gyrus, where both ER-α and ER-β coexpression is found in ~80% of proliferating cells (34, 40). The generation of new neurons in the dentate gyrus via estrogen signaling through ER-α may also be important for the maintenance of hippocampal function and for adequate control of the HPA axis (13).

Estradiol signaling via ER-α also cooperates with vesicular glutamate transporter 2 (VGluT2)-expressing neurons in the amygdala and the VMN (40). Coexpression of ER-α and VGluT2 mediates the effects of estrogen on the rat medial amygdala, the center of emotional responses to fear and anxiety (27). Social recognition and anxiety in female rats are regulated by ER-α localized in this region (47).

The effect of CR on preservation of ER-α in the limbic system appears to promote neural plasticity and fear regulation. The prolonged neural plasticity and fear regulation in the limbic system are consistent with the need to battle neurodegeneration and mood instability that are common in old age. In extending life span, not only does CR prolong reproductive functions and stress response abilities by preserving ER-α

function in the hypothalamus, but ER-α preservation in the limbic system prolongs neural plasticity and emotional regulation.

ER-α Signaling and Its Effect on Aging: Effect of CR

In animal models of aging and CR, alterations in hypothalamic sensitivity to peripheral hormones, particularly estrogen, are known to influence the reproductive capabilities, maturation, and senescence of an organism. Recent studies have shown that the age-related reduction of total cells within the ARC and POA of the hypothalamus, as well as ER-α immunoreactive cell populations in those regions, contribute to normal aging (39). CR has been shown to have a protective effect against this age-related cell loss by maintaining ER-α-expressing cells (55). On average, CR has been shown to attenuate the extensive ER-α immunoreactive cell loss by 31%, helping maintain reproductive potential (55). This allows for the maintenance of hormonal sensitivity in the hypothalamus, thus aiding continued estrogen signaling and extending life span (56).

Along with significantly slowing the onset of aging phenotypes, CR has also been shown to reduce the onset of oocyte loss accompanied with menopause and, in turn, decelerate neural, reproductive aging processes as well as many other physiological systems (39). In addition, protective capabilities of CR in the maintenance of ER-α-expressing cells seem to have profound evolutionary implications. According to the hypothalamic dysregulation hypothesis, both puberty and aging are the products of alterations in hypothalamic sensitivity to specific hormones like ER-α. CR extends reproductive life span, which is considered the central evolutionary determinant of total enhanced longevity (15, 18).

Despite the early experimental design problems of equating ad libitum diet as normal diet long-term CR has been shown to extend the life and health spans in laboratory-raised and -housed organisms ranging from nonhuman primates and rodents to invertebrates. However, results of studies on hyperphagic, caged mammals are discordant with current physical activity and dietary recommendations for healthful living for humans. Hence, the absence of controlled, randomized prospective studies on humans limits interpretation of CR studies on nonhuman species for human populations (16, 22, 33, 35, 49).

Given the effects of CR on ER-α expression in various hypothalamic nuclei, studies have sought for mechanistic links between CR and ER-α expression. First, peroxisome proliferator activated receptor gamma coactivator-1α (PGC-1α) is known to be a key regulator in glucose metabolism, cellular respiration, energy production, and adaptive thermogenesis (9, 31, 54). Studies have shown that CR induces PGC-1α leading to mitochondrial biogenesis and increased life-span (34, 42). In vitro CR models, shows that CR elevates PGC-1α expression and mitochondrial function (32). Furthermore, calorie-restricted human subjects have increased levels of PGC-1α (30). This is concomitant with studies that show PGC-1α as mediator of AMP-activated protein kinase (AMPK), which regulates mitochondrial biogenesis (30).

PGC-1α is also a coactivator of ERα on estrogen-responsive element (ERE)-driven transactivity through the interaction of carboxyl terminus of PGC-1 with the ER-α hinge domain (3, 28). Mitochondria biogenesis and energy production are increased through the overexpression of PGC-1α. Several studies report that PGC-1α can ameliorate mitochondrial dysfunctions that are caused by certain age-related diseases including neurodegenerative diseases (11). In vitro studies have shown that PGC-1α elevates the estradiol-dependent transactivity of ER-α (38). Thus PGC-1α seems to be the mechanism by which CR induces ER-α expression, which leads to well-known neuroprotective effects of CR in aging.

CR and Longevity in Humans

Having established the plausible mechanism between CR, PGC-1α, and ER-α in aging, studies have shown that CR's effects on aging extends to humans as well (14, 50). CR studies on humans have shown less age-associated phenomena such as myocardial stiffness and autonomic dysfunction, as well as downregulation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/forkhead box O (FOXO) and inflammatory pathways in skeletal muscle (2). Consistent with the slow aging rodent models, humans on CR also exhibit signs of slower aging with low levels of inflammation due to low C-reactive protein and tumor necrosis factor-alpha (TNF-α), decreased serum triiodothyronine levels, and a more elastic and healthier left ventricle (4). Considering that aging is marked by the development of disease, long-term CR in humans has been shown to also support "slow aging" by increasing the healthy life span and lowering the risk of many age-related diseases such as cardiovascular disease, cancer, diabetes, neurodegenerative diseases, and several autoimmune disorders (14). Reduction of body weight by CR in humans has been shown to improve glucose tolerance and increases insulin sensitivity, while also decreasing the risk of developing Type 2 diabetes, hypertension, coronary heart disease, dementia, breast cancer, prostate cancer, and colon cancer (54). Similarly, CR has also been proven to also protect normal cells and organs from toxic conditions while promoting the death of cancer cells (4). The role of ER-α, especially in breast cancer progression, has been shown as drug-resistant cancer cells still have ER-chromatin occupancy but acquired differential ER-binding profiles modulated by progesterone receptor (PR), which can be linked to the effects of CR on cancer (37, 43). Prolonged CR in humans also reduces two biomarkers of longevity, which are fasting insulin level and body temperature.

One novel insight into the effects of long-term CR in humans stems from studies of the Okinawan population of Japan who are renowned for their reduced morbidity and mortality (14). While their diet is similar to those under CR, an uncontrolled variable in the study was the level of physical activity by Okinawans. Despite the difference in physical activity level, the Okinawan life style resulted in a 50% lower death rate between ages 60–64 yr than Americans, and a 30–40% lower death rate from heart disease, stroke, and cancer than in other regions in Japan itself (14). The extension of health and life spans seen in CR animal experiments mimics the effects seen in the Okinawans (51–53).

Results from the Biosphere 2 experiment also proved the health benefits of the CR diet. In 1991, eight subjects entered Biosphere 2, a closed environment in an Arizona desert region. There, those individuals maintained a CR diet for the duration of 2 yr, and their physiological and biochemical responses were monitored for 18 mo after returning to their normal diets

(50). During CR, the eight individuals experience a lower metabolic rate, body temperature, systolic and diastolic blood pressure, and thyroid hormone levels. Despite weight loss, the participants of this study remained in excellent health and maintained high levels of physical and mental activity throughout the entire 2 yr period (50). Regrettably, the investigators did not include the cohort in which caloric control or physical fitness were manipulated with exercise training; nonetheless, they concluded that healthy nonobese humans on a CR diet show physiological, hematological, hormonal, and biochemical changes that are similar to those seen in in rodents and nonhuman primates under CR (50).

In another study done by the Caloric Restriction Society, 18 volunteers practiced the CR diet for 3–15 yr, consuming 1,112–1,958 kcal/day, while age-matched and height-matched controls consumed 1,976–3,537 kcal/day (14). The subjects showed significant reductions in blood glucose levels, insulin levels, and blood pressure (17). The 18 test subjects also demonstrated reduced total cholesterol, low-density lipoprotein (LDL), and triglycerides and increased high-density lipoprotein (HDL) compared with the controls (17). Another 6 mo randomized clinical trial of overweight or obese premenopausal women that fasted for 2 nonconsecutive days per week showed similar results. In both studies, the CR group showed a reduction of C-reactive protein and about a 40% reduction of carotid artery intima-media thickness, thereby proving a reduced risk of atherosclerosis and cardiovascular disease (17).

Although CR may have the ability to increase the health span in animal model systems, it does not come without risks for humans. Results from the 1950 Minnesota Starvation Study in which 36 physically and psychologically healthy men underwent a 50% of normal dietary intake CR for 6 mo showed that some individuals participating in a CR diet may experience loss of libido, hypotension, menstrual irregularities, infertility, and irritability (14). During CR, low estrogen levels could also potentially lead to bone thinning or osteoporosis, while hormonal changes may lead to slower wound healing (14). Further, obesity resulted in the post-CR period of the Minnesota Starvation Study. For these reasons, the extent and long-term effects of CR should be strongly considered when further studying CR in humans.

Summary

In humans and rodent models, ER-α signaling occurs in the different regions of the hypothalamus, namely the ARN, POA, VMN, AVPV, PVN, SON, SCN, as well as the amygdala and hippocampus of the limbic system. Throughout development, nuclei of the hypothalamus function to maintain homeostasis sensitivity of hypothalamic neurons to estradiol, affecting growth and development and, ultimately, health and longevity of the organism. In each hypothalamic and limbic brain region, ER-α signaling plays a unique role with respect to the different function of the nuclei/region itself. First, ER-α in the hypothalamus, especially within the ARN and POA, controls reproductive functioning by upregulating GnRH production. Second, ER-α signaling centralized in the ARN, POA, VMN, and AVPV nucleus modulates sexual and social behaviors. Third, ER-α signaling in the POA is crucial to maintain maternal behavior in females and paternal acceptance to parental care. Fourth, within the SON and PVN, the ER-α signaling also protects neurons from osmotic stress, which in turn strengthens the HPA axis in handling stress. Fifth, within the SCN, ER-α signaling is responsible for the regulation of clock genes such as period circadian clock 2 (PER2) and maintenance of normal circadian rhythm. Sixth, in the limbic system, ER-α expression contributes to learning and memory through neurogenesis, regulation of anxiety, neuroprotective effects against glutamate, $FeSO_4$, amyloid-β peptide toxicity, and glucose deprivation. Mechanistically, PGC1-α, AMPK, PI3K, AKT, and FOXO have been implicated in mediating CR and ER-α effects in promoting longevity.

Considering the critical role of ER-α expression in these regions, disruption in ER-α sensitivity in any of these aforementioned areas of the hypothalamus and limbic system would be expected to produce significant effects to the health and functioning of the organism itself, greatly contributing to the overall longevity. As an evolutionary response to changing environments, the effects of ER-α signaling have been positively selected to maximize their life span to create viable offspring at the most appropriate time. In this paper, we articulate the hypothalamic deregulation hypothesis by which ER-α signaling and hypothalamic and limbic hormonal sensitivity affect mammalian life span under specific stress conditions, such as CR. In previous studies, CR has been shown to maintain ER-α-expressing cells and to provide the stimulus to impede changes in hormonal sensitivity, extending the reproductive life span and consequently lengthening the total mammalian life span by delaying senescence. ER-α signaling maintains the normal functioning of the aforementioned hypothalamic and limbic brain regions and consequently allows for the prolonging of healthier conditions for the organism.

Currently, minimal research has been done on the effects of CR within individual nuclei, as well as the evolutionary implications for each of their individualized responses. In addition, lack of randomized, controlled prospective studies limits translation of data from rodents and other animals to the human condition, particularly because of the recommendations for human diet and nutrition based on quality good clinical practice promoted by several professional medical and health-promoting organizations. Nonetheless, ER-α regulation in the regions of the hypothalamus and limbic system in relation to CR continues to be an important area of research to further understand the processes of normal aging and the implications for improving human wellbeing.

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DISCLOSURES

No conflicts of interest (financial or otherwise) are declared by the author(s).

AUTHOR CONTRIBUTIONS

A.M.G., G.E., R.B., A.P., M.M., and S.A.G. compiled research publications; A.M.G., G.E., R.B., S.A.G., and G.A.B. interpreted results of experiments; A.M.G. prepared figures; A.M.G., G.E., M.M., and S.A.G. drafted manuscript; A.M.G., G.E., R.B., A.P., M.M., S.A.G., and G.A.B. edited and revised manuscript; A.M.G., G.E., R.B., A.P., M.M., S.A.G., and G.A.B. approved final version of manuscript.

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